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EFFECTS OF HEXAMETHONIUM AND HYOSCINE ON THE DRUG-INDUCED DEPOLARIZATION OF ISOLATED SUPERIOR CERVICAL GANGLIA

BY

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Measurement of ganglionic depolarization *in vitro* using a moving-fluid external recording electrode has been described previously by Pascoe (1956), Mason (1962) and Brown (1966). The present paper describes the effects of hexamethonium and hyoscine on the depolarization of isolated superior cervical ganglia produced by carbachol, acetylcholine, nicotine, muscarine and methacholine. Most of the experiments were done using kitten ganglia. These were employed instead of adult cat ganglia for reasons described previously (Brown, 1966). A few experiments were also performed using isolated rat ganglia.

METHODS

Superior cervical ganglia were isolated from 6- to 10-week-old kittens (anaesthetized with 35 mg/kg of sodium pentobarbitone intraperitoneally), or from 200 to 250 g rats (anaesthetized with 1.5 g/kg of urethane intraperitoneally). The ganglia were mounted in a 50 ml. bath of Krebs' solution at room temperature (20.5 to 22° C), previously equilibrated with 95% oxygen/5% carbon dioxide mixture. Ganglionic potential changes were recorded as described previously (Brown, 1966).

Immediately after isolation a large positive demarcation potential (1 to 3 mV) existed between the surface of the ganglion and the end of the postganglionic nerve trunk. To facilitate the measurement of small changes of ganglionic potential, using high amplification, this demarcation potential was allowed to subside before beginning the experiment. Since this required several hours, it was usual to leave the preparation overnight between isolation and experimentation, mounted on the electrodes and immersed in oxygenated Krebs' solution at 4° C. This procedure did not noticeably affect the response to depolarizing drugs, nor was transmission affected.

A sweep of the moving-fluid electrode gave a picture of the potential difference between any point on the surface of the preparation and the end of the postganglionic trunk (to which the reference electrode is attached) (see records in Figs. 1, 2, 4, 6 and 10). Ganglionic depolarization was indicated by an upward (negative) shift of the record from that given by a control sweep. Normally, depolarization was measured at the region of the preparation showing the largest negative shift: this was usually around the preganglionic end of the ganglion, and was constant in each preparation.

Ganglionic potential changes were recorded with a direct-coupled pre-amplifier giving a magnification on the oscilloscope screen of up to 200 μV/cm, and were measured to the nearest 10 μV. It was possible to discern drug-induced potential changes of the order of 20 to 50 μV from rapid fluctuations or slow spontaneous drifts of inter-electrode potential (Fig. 8,*c*). Amplifier drift during the 5 sec or so required to make a sweep with the moving-fluid electrode was less

than 20 μV. Records of potential distribution were taken at intervals before and after adding drug to the bath, and the change in ganglionic potential plotted against time (see Fig. 1). With kitten ganglia, exposure to depolarizing agent for about 10 min was usually required before full depolarization was obtained, and 1 hr washing with 3 or 4 changes of Krebs' solution was necessary to allow complete recovery between doses. With rat ganglia, 4 min exposure to drug and 45 min washing between doses sufficed. Blocking agents were added to the bath 15 min before application of depolarizing agent.

The following drugs were used: aceteylcholine chloride, carbachol chloride (carbaminoylcholine), methacholine chloride (acetyl-β-methylcholine), nicotine hydrogen tartrate, dl-muscarine iodide (synthetic), hexamethonium bromide, hyoscine hydrobromide, atropine sulphate. The drugs were added to the bath dissolved in 0.5 ml. or less of 0.9% saline. Doses are given throughout as bath concentrations of the salt in g/ml.

RESULTS

Depolarization of isolated kitten ganglia

Carbachol.—The ED50 depolarizing concentration of carbachol on the isolated kitten ganglion is about 10^{-5} g/ml. Brown, 1966; see also Fig. 8,*a*). This concentration was used in all experiments described below, except where otherwise stated. It produced between 0.5 and 1.4 mV depolarization, and its effect on any one preparation was constant within 10% over 5 or 6 applications when 1 hr was allowed between doses (three control experiments).

Hexamethonium, in concentrations up to 10^{-3} g/ml., reduced, but did not abolish, the depolarization elicited by 10^{-5} carbachol (Fig. 1, Table 1). Between 25 and 45% of the initial depolarization remained in the presence of 10^{-3} hexamethonium. This incomplete block was not the result of insufficient hexamethonium, because the amount of block obtained with 10^{-3} hexamethonium was no greater than that seen with 10^{-4} hexamethonium. Further, the depolarization produced by 10^{-5} carbachol in the presence of 10^{-3} hexamethonium was not augmented by increasing the concentration of carbachol to 10^{-4} g/ml.

TABLE 1

EFFECTS OF HEXAMETHONIUM ON DRUG-INDUCED DEPOLARIZATION OF ISOLATED KITTEN SUPERIOR CERVICAL GANGLIA

Values give % reduction (−) or increase (+) of response. Each set of values refers to a single experiment

			Concentration of hexamethonium (g/ml.)			
Depolarizing agent	Concn. (g/ml.)	Expt. no.	10^{-5}	10^{-4}	10^{-3}	10^{-4} + hyoscine 10^{-6}
Carbachol	10^{-5}	2	−48	−71	− 76	
	10^{-5}	17	−42	−58	− 60	
	10^{-5}	11		−67	− 73	−100*
	10^{-5}	14			− 75	− 94*
	10^{-5}	18		−75	− 72	− 96
	10^{-5}	23		−50	− 55	− 89
Acetylcholine	10^{-3}	21	−48	−76	− 80	− 94
	10^{-3}	23		−79	− 82	− 97
Nicotine	10^{-5}	17	−86	−97		
	10^{-5}	24	−69	−95	−100	
Muscarine	10^{-6}	18		+ 5	± 0	
	10^{-6}	24	− 5	− 8	− 3	
Methacholine	10^{-3}	7	−10	−20	− 30	
	10^{-3}	9			− 10	

* Hexamethonium 10^{-3} + hyoscine 10^{-6}.

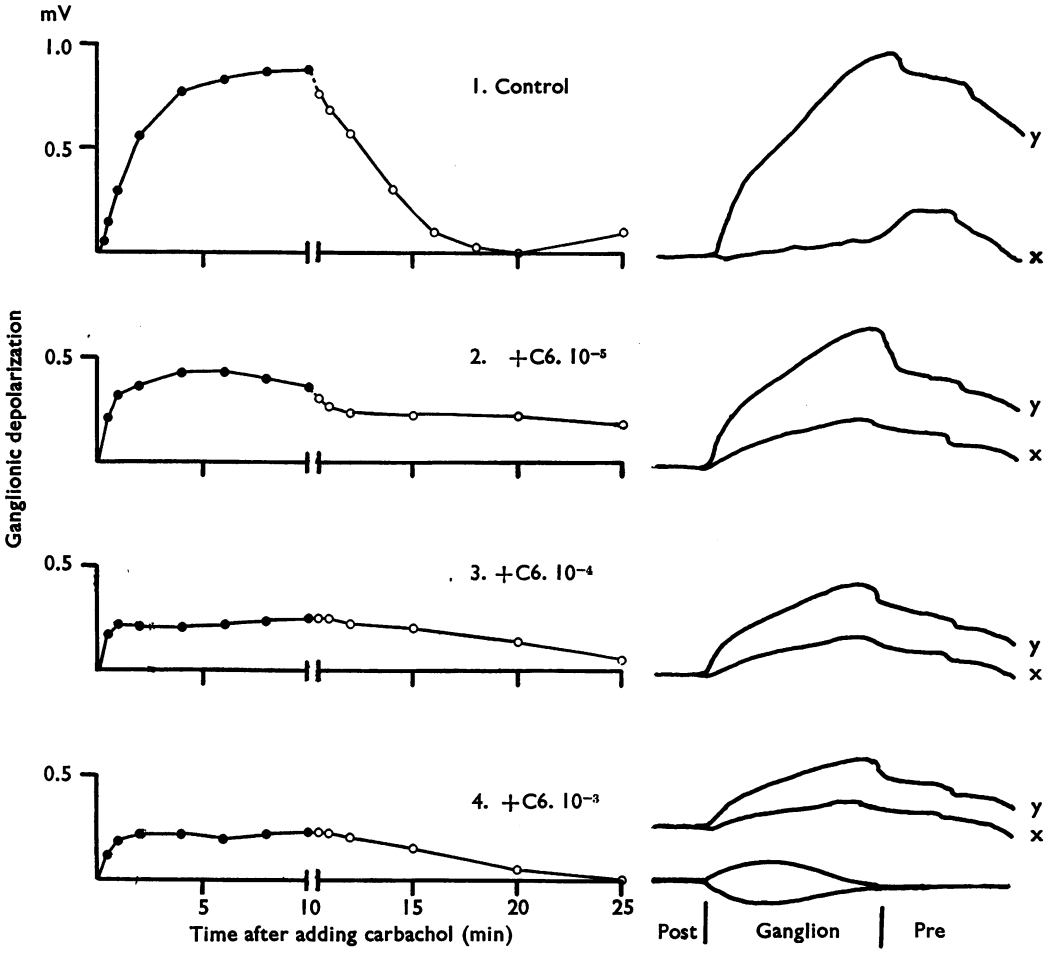


Fig. 1. Effect of 3 concentrations of hexamethonium (C6, concentrations in g/ml.) on the depolarization of an isolated kitten superior cervical ganglion produced by 10^{-5} g/ml. of carbachol. The graphs on the left show the time-course of the development of ganglionic depolarization after addition of carbachol to the bath (filled symbols) and its subsidence (open symbols) after washing (at break in abscissae)—1, in the absence of hexamethonium, and 2, 3 and 4, 15 min after adding 10^{-5}, 10^{-4} and 10^{-3} g/ml. of hexamethonium respectively. Tracings of oscilloscope records obtained with the moving-fluid electrode before (x) and 10 min after (y) each application of carbachol are shown on the right. These records give the potential difference between the region of the preparation in contact with the moving electrode and the cut end of the post-ganglionic nerve trunk. The moving electrode was swept down the preparation from post-ganglionic to preganglionic nerves, and the electron beam on the oscilloscope was simultaneously deflected from left to right. The position of the moving electrode on the preparation is indicated below the records. Calibration of the potential change is the same as that given on the ordinates of the graphs. Carbachol-induced depolarization was measured by the maximal upward (negative) displacement of record (y) from record (x) in each case.

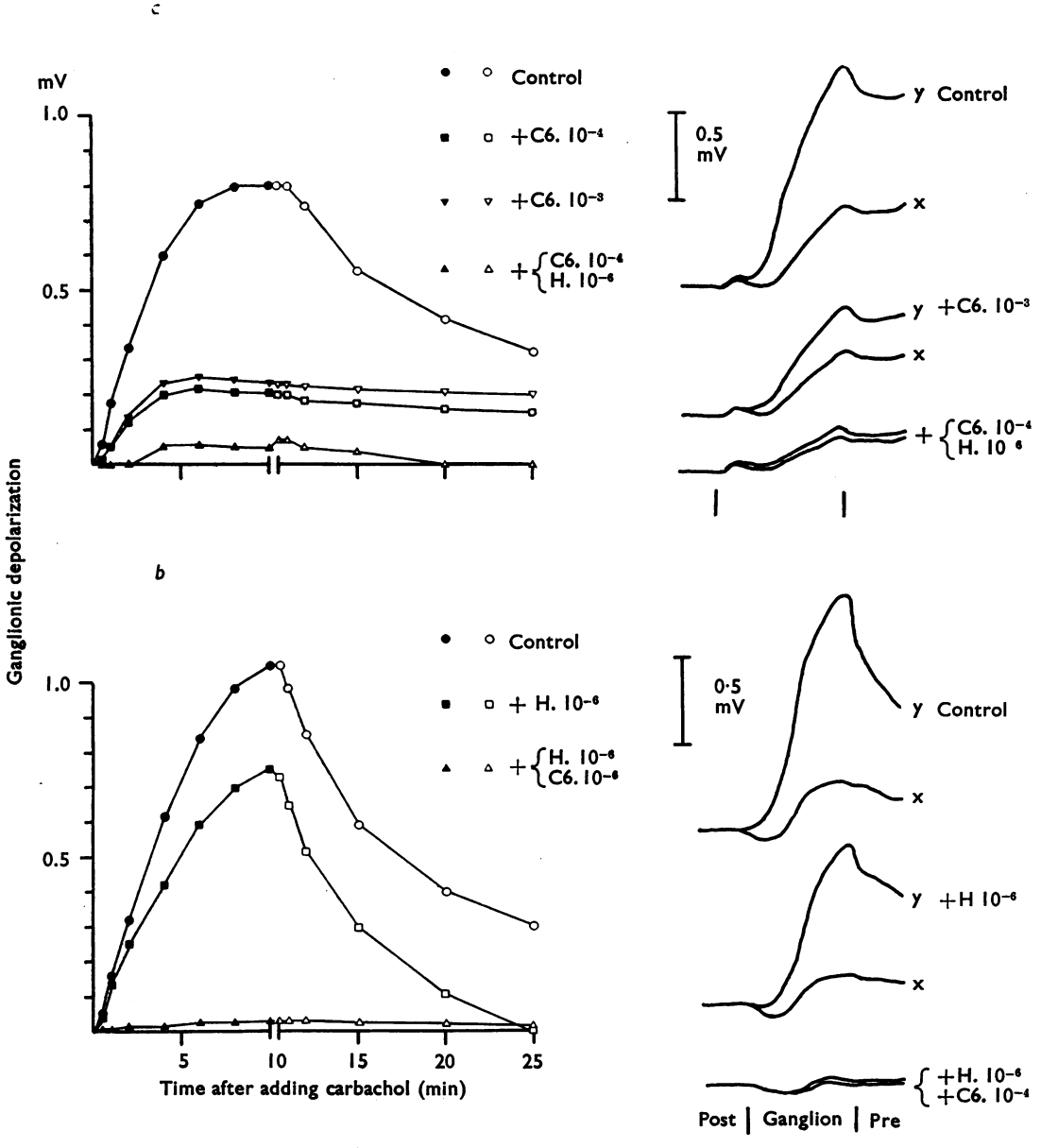


Fig. 2. Effects of hexamethonium (C6) and hyoscine (H), separately and in combination, on the depolarizing action of 10^{-5} g/ml. of carbachol on two isolated kitten ganglia. Time-courses of ganglionic depolarization and recovery are shown on the left, and tracings of oscilloscope records (negativity upwards) obtained before (x) and 10 min after (y) each application of carbachol are illustrated on the right.

The residual depolarizing action of carbachol in the presence of hexamethonium was blocked or substantially reduced by the further addition of 10^{-6} hyoscine (Fig. 2,*a*; Table 1).

In the absence of hexamethonium, the effect of 10^{-5} carbachol was reduced by 10^{-6} hyoscine alone by up to 30% (Fig. 2,*b*; Table 2). The response to a lower dose (2×10^{-6})

TABLE 2

EFFECTS OF HYOSCINE (H) AND ATROPINE (A) ON DRUG-INDUCED DEPOLARIZATION OF ISOLATED KITTEN SUPERIOR CERVICAL GANGLIA

Values give % reduction (−) or increase (+) of response. Each set of values refers to a single experiment

Depolarizing agent	Concn. (g/ml.)	Expt. no.	H/A	Concentration of hyoscine or atropine (g/ml.) 10^{-6}	10^{-5}	10^{-4}
Carbachol	10^{-5}	9	A	− 7	−43	−80
	10^{-5}	15	H	− 12		
	10^{-5}	16	H	− 32	−26	−29
	2×10^{-6}	20	H	− 59		
Acetylcholine	10^{-3}	22	H	+ 5	− 5	
Nicotine	10^{-5}	19a	H	+ 2	± 0	−20
		19b	A	− 8	−53	−96
	10^{-5}	24	H	− 5		
Muscarine	10^{-6}	20	H	−100		
	10^{-6}	24	H	−100		
Methacholine	10^{-3}	8	A	− 60	−87	
	10^{-3}	10	A	− 67		
	10^{-3}	13	H	− 58	−70	

of carbachol appeared to be depressed more strongly by hyoscine (Fig. 6). Increasing the concentration of hyoscine to 10^{-4} did not greatly add to the block. Subsequent addition of hexamethonium led to complete block (Fig. 2,*b*). The effect of atropine differed from that of hyoscine in that increasing the concentration of atropine from 10^{-6} to 10^{-4} led to a progressive increase in the amount of block (Table 2). A comparable difference between the effects of hyoscine and atropine on the response to nicotine was seen (see below).

On removal of carbachol from the bath, the depolarization of the isolated kitten ganglion subsided slowly (see Fig. 8), with only occasional evidence of the after-hyperpolarization seen with rat ganglia (see Figs. 1 and 5, and compare with Fig. 7). In the presence of hexamethonium, repolarization was slower than that seen in controls (Fig. 1). This was similar to the effect obtained on reducing the concentration of applied carbachol (Fig. 8,*a*). In contrast, after hyoscine the recovery of the polarity was, if anything, more rapid than the normal recovery rate (Figs. 2,*b* and 6).

Neither hexamethonium nor hyoscine affected the distribution along the preparation of the carbachol-induced depolarization (see oscilloscope records in Figs. 1, 2 and 6). Thus, measurements of depolarization taken at regions other than those yielding maximal potential shifts gave results similar to those described above.

Acetylcholine.—In the absence of anticholinesterase agents, a concentration of acetylcholine of the order of 10^{-3} g/ml. was necessary to evoke a large depolarization of the isolated kitten ganglion. Comparison of the responses of a single preparation to

carbachol and acetylcholine (Fig. 3) indicated that the effect of acetylcholine was reduced to a greater extent by hexamethonium than was that of carbachol. However, even after 10^{-3} hexamethonium acetylcholine produced about 20% of the initial depolarization. This residual depolarization was considerably reduced by the further addition of 10^{-6} hyoscine. Hyoscine alone did not greatly affect the depolarizing action of acetylcholine (1 experiment, Table 2).

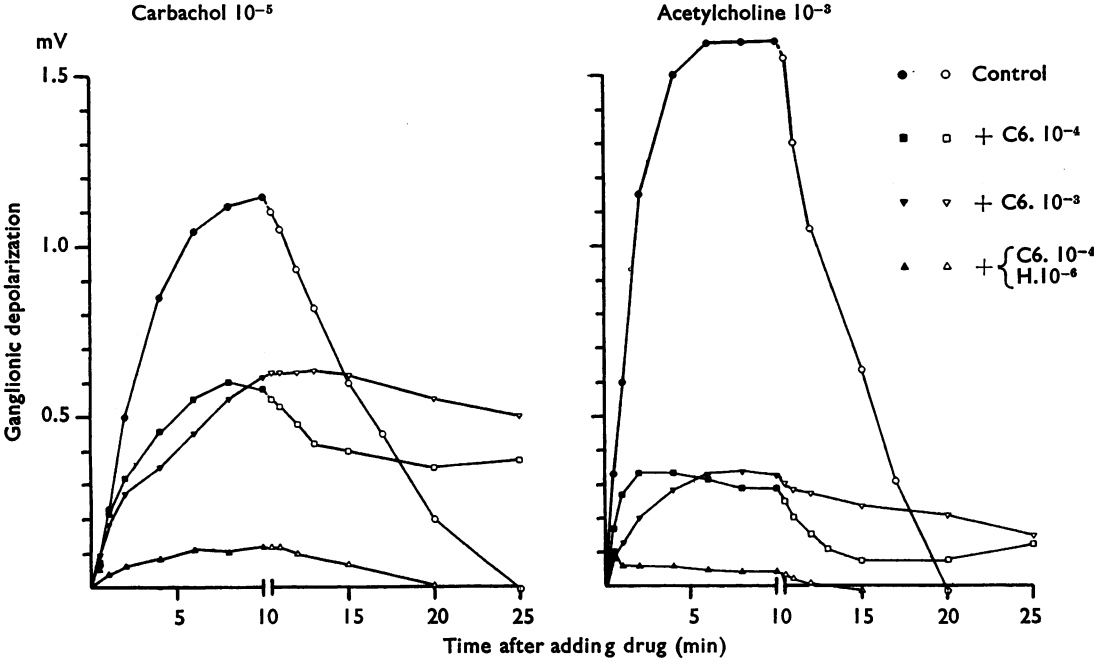


Fig. 3. Graphs showing the effects of hexamethonium (C6) and hexamethonium with hyoscine (H) on the depolarizing actions of 10^{-5} g/ml. of carbachol and 10^{-3} g/ml. of acetylcholine, recorded in the same isolated kitten ganglion.

Nicotine.—The depolarization obtained with 10^{-5} nicotine approximated in size to that seen following application of 10^{-5} carbachol to the same preparation, but the nicotine-induced depolarization developed more slowly and subsided more slowly on washing than did that produced by carbachol (Fig. 4). The spatial distribution of the nicotine depolarization differed from that seen with carbachol: the nicotine depolarization was not restricted to the ganglion, but spread some way down the postganglionic nerve trunk (see records in Fig. 4).

The response to 10^{-5} nicotine was substantially reduced by 10^{-5} hexamethonium, and was blocked completely by 10^{-4} hexamethonium (Fig. 4). This was in marked contrast to the incomplete blocking action of 10^{-4} hexamethonium on the response of the same preparation to carbachol.

The action of nicotine was not materially affected by hyoscine in concentrations below 10^{-4}, but was reduced to half by 10^{-5} atropine, and blocked by 10^{-4} atropine (Table 2; 1 experiment).

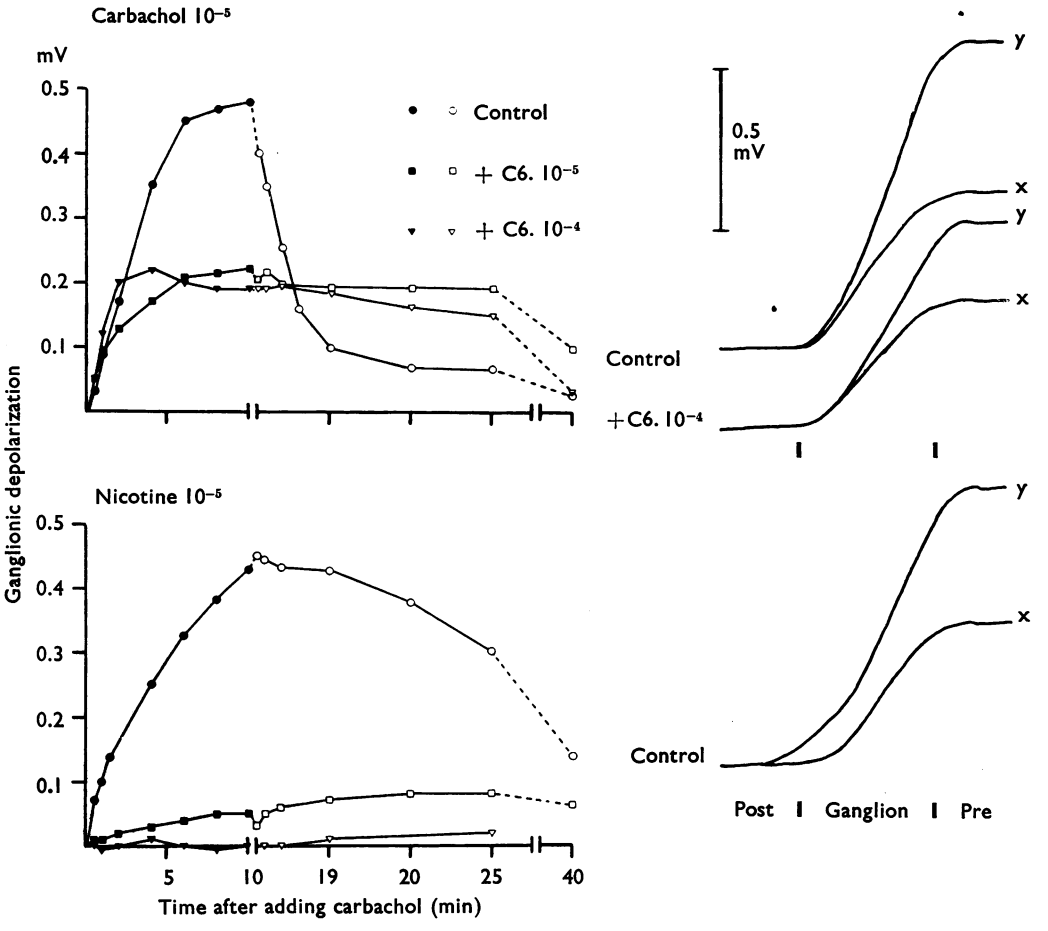


Fig. 4. Comparison of the effects of hexamethonium (C6) on the depolarizing actions of 10^{-5} g/ml. of carbachol and 10^{-5} g/ml. of nicotine recorded in the same isolated kitten ganglion. Time-courses of ganglionic depolarization and recovery are shown on the left, and tracings of oscilloscope records (negativity upwards) obtained before (x) and 10 min after (y) addition of the depolarizing agents are shown on the right.

Muscarine.—Addition of dl-muscarine iodide produced a small depolarization of the kitten ganglion (200 to 300 μV ; 3 experiments) which did not increase within the dose-range of 10^{-7} to 10^{-5} g/ml. (Fig. 5). This was much less than the depolarization evoked by 10^{-5} carbachol, but was similar to that part of the response to carbachol which remained after 10^{-3} hexamethonium, and which was blocked by hyoscine. The spatial distribution of the muscarine-induced depolarization along the preparation was similar to that seen with an equi-effective concentration of carbachol (Fig. 6).

The action of muscarine was not modified by 10^{-3} hexamethonium (Fig. 5), but was abolished by 10^{-6} hyoscine (Figs. 5 and 6).

Methacholine.—Concentrations of 10^{-4} to 10^{-3} methacholine produced a small ganglionic depolarization. With 10^{-3} methacholine this ranged in size from 30 to 360 μV.

—i.e., between 3 and 25% of the depolarization produced in the same preparations by 10^{-5} carbachol (7 experiments). The spatial distribution of the depolarization produced by methacholine resembled that obtained with carbachol (Fig. 11).

The depolarizing action of methacholine was reduced slightly by 10^{-3} hexamethonium. It was substantially reduced, but never abolished, by 10^{-6} hyoscine or atropine (Tables 1 and 2).

Concentrations of methacholine below 10^{-4} produced a positive ganglionic potential change (see below and Fig. 11).

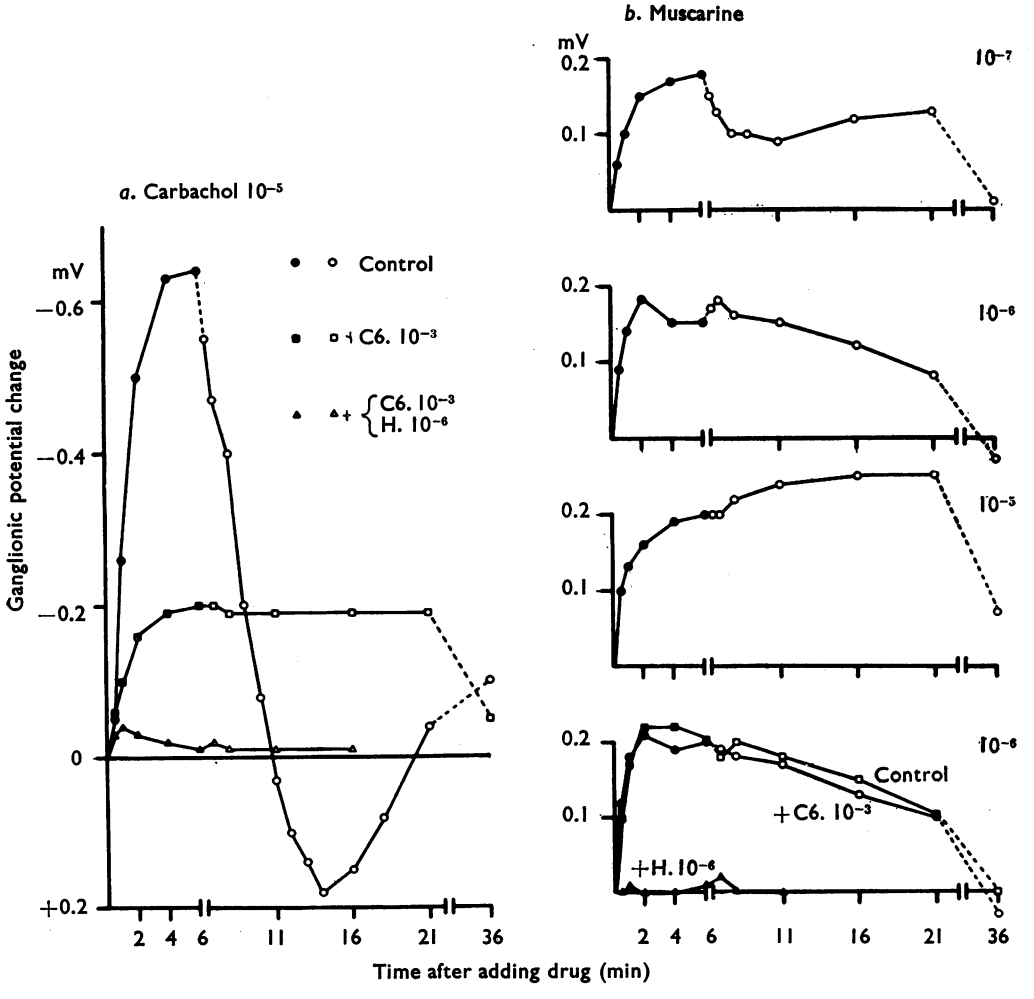


Fig. 5. Comparison of the depolarizing actions of carbachol and muscarine in a single isolated kitten ganglion. (*a*) Responses to 10^{-5} g/ml. of carbachol before and after adding hexamethonium (C6) and hexamethonium with hyoscine (H). (*b*) Effects of 10^{-7}, 10^{-6} and 10^{-5} g/ml. of synthetic dl-muscarine iodide, and (lowest graph) of 10^{-6} g/ml. of muscarine before and after adding hexamethonium and hyoscine separately.

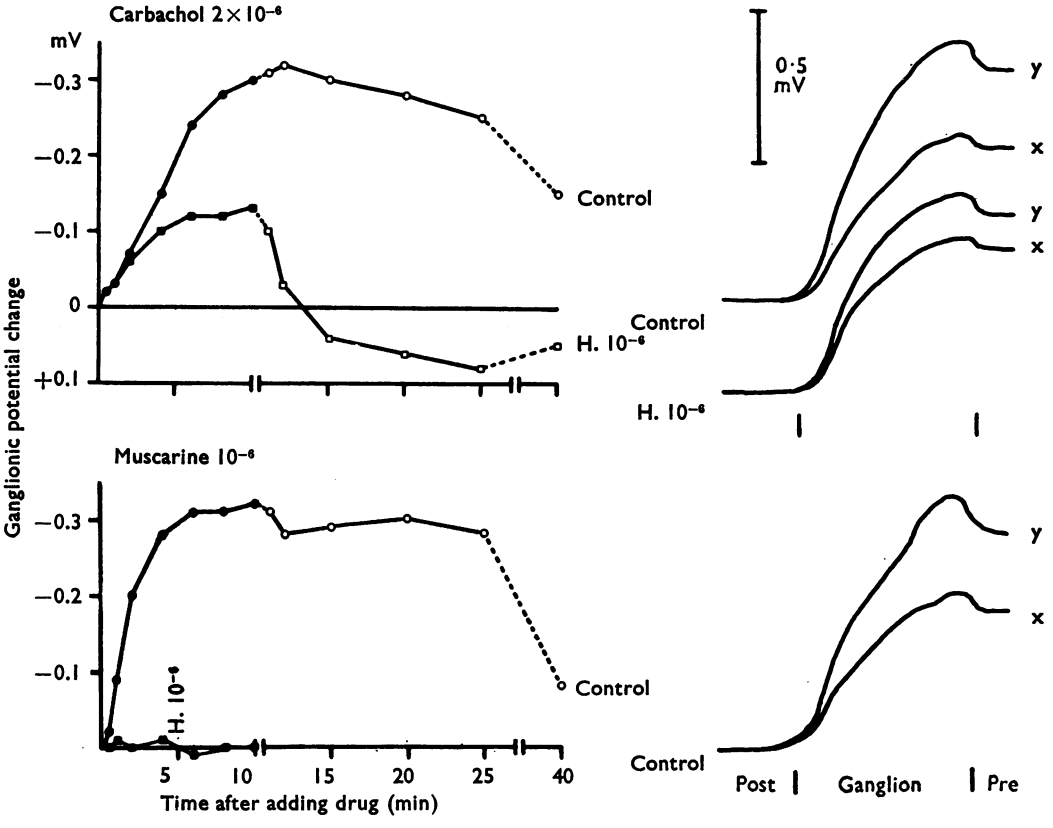


Fig. 6. Effects of 10^{-6} g/ml. of hyoscine (H) on the depolarization of a single isolated kitten ganglion produced by equi-effective concentrations of carbachol and muscarine. Graphs on the left show the time-courses of the changes of ganglionic potential evoked by 2×10^{-6} g/ml. of carbachol and 10^{-6} g/ml. of muscarine. Tracings of oscilloscope records (negativity upwards) obtained before (x) and 10 min after (y) addition of the two depolarizing agents are shown on the right.

Depolarization of isolated rat ganglia

The effects of hexamethonium on the responses of 2 isolated rat ganglia to 10^{-5} carbachol and 2 ganglia to 3.2×10^{-4} acetylcholine were investigated (Fig. 7).

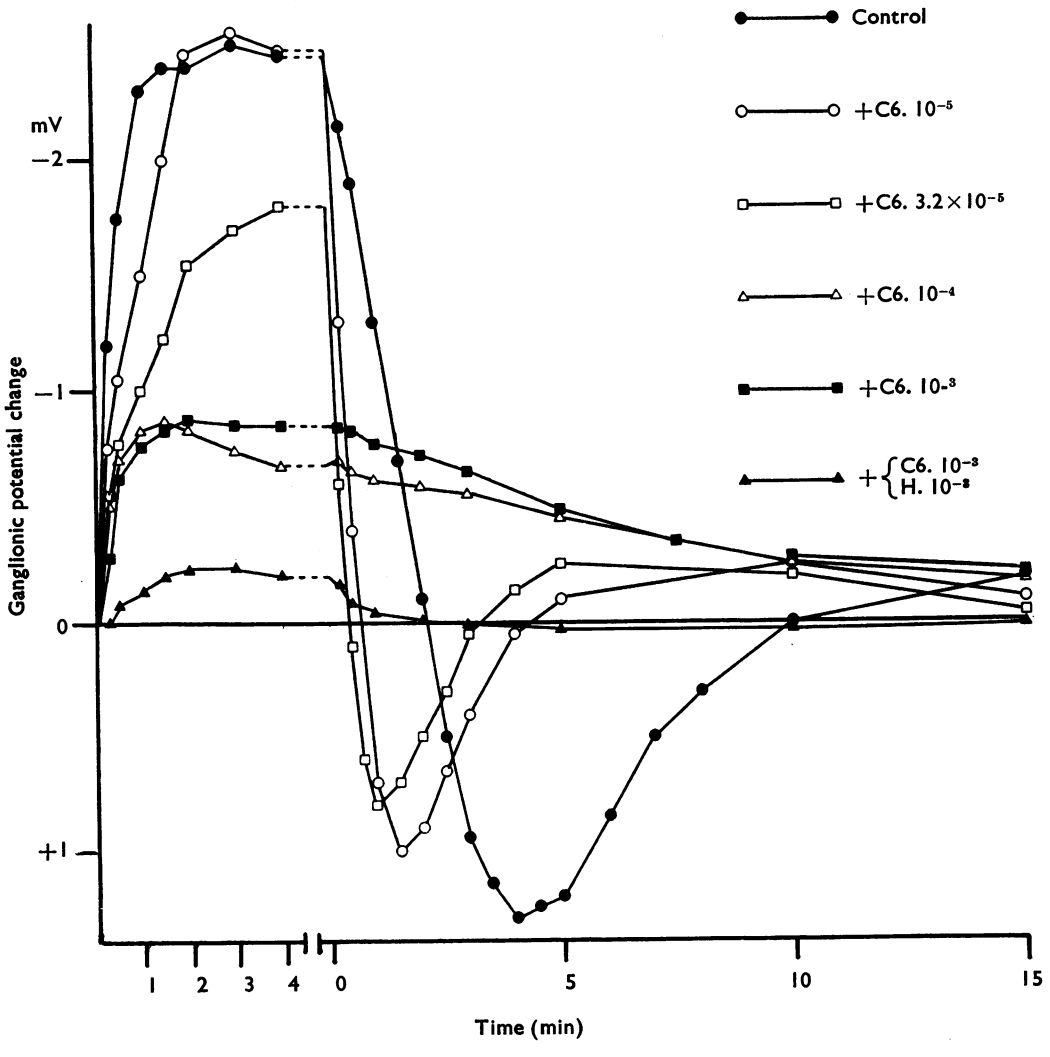
Concentrations of hexamethonium of 10^{-5} or above reduced the depolarization produced by either carbachol or acetylcholine. Hexamethonium also accelerated the development but reduced the size of the after-hyperpolarization obtained on removing the carbachol. This effect is similar to that obtained by reducing the concentration of depolarizing agent (see Fig. 1 in Brown, 1966). In the presence of large concentrations of hexamethonium, the development of depolarization after addition of acetylcholine showed a complex, biphasic time-course (Fig. 7,*b*). This was confirmed in the second experiment. It was not seen with carbachol in the presence of hexamethonium (Fig. 7,*a*),

nor with low concentrations of acetylcholine in the absence of hexamethonium (Brown, 1966).

As with kitten ganglia, hexamethonium (10^{-3}) did not completely block the depolarizing action of either carbachol or acetylcholine, but reduced the response to about 70% of the control level. The residual response was further reduced by 10^{-6} hyoscine. The effect of hyoscine on the hexamethonium-resistant response of the rat ganglion to carbachol was greater than its effect on the equivalent response to acetylcholine. Hyoscine did not modify the biphasic nature of the depolarizing action of acetylcholine in the presence of hexamethonium.

Closely similar results were obtained in 2 chronically denervated rat ganglia.

Fig. 7a *a*. Carbachol 10^{-5}



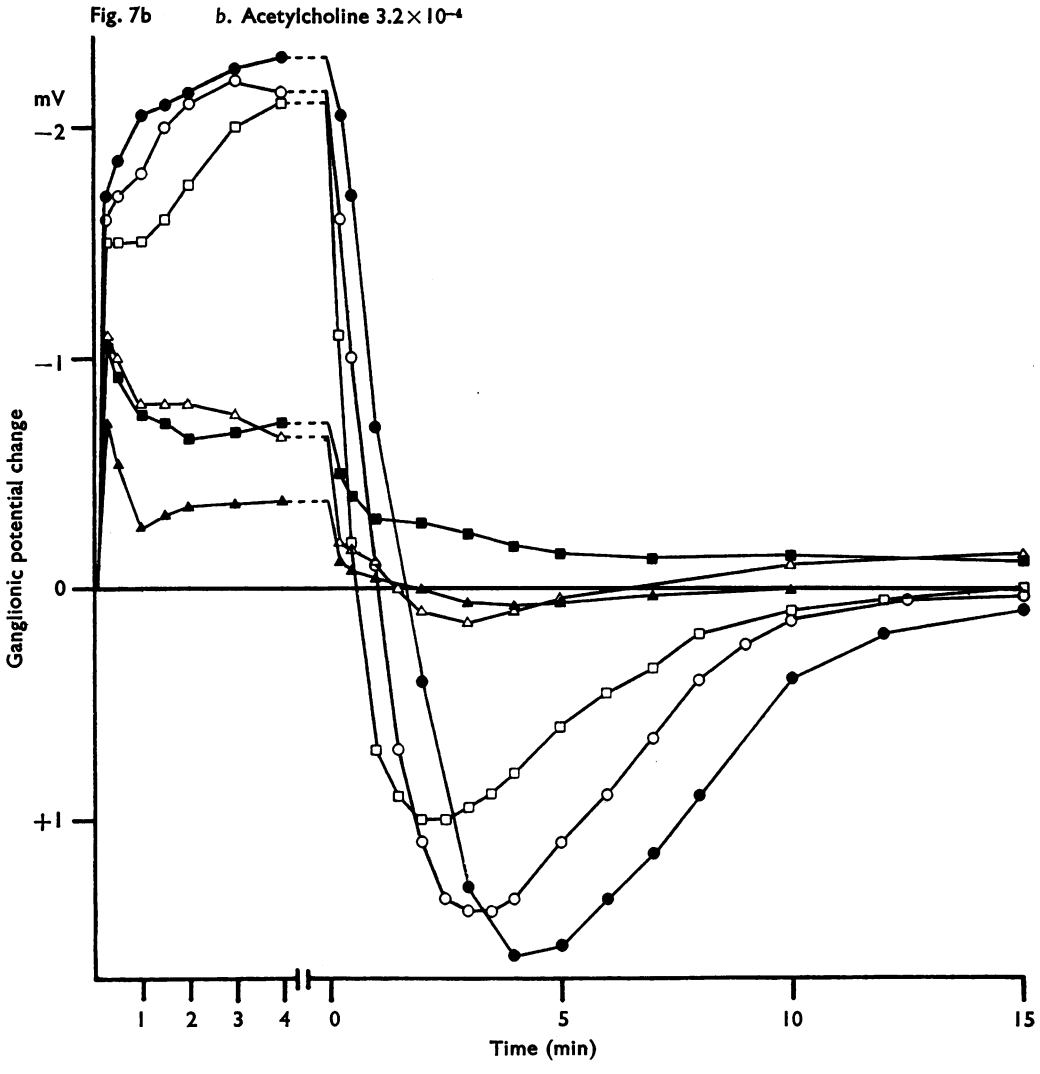


Fig. 7. Effects of increasing concentrations of hexamethonium (C6) and of a combination of hexamethonium with hyoscine (H) on the depolarizing actions of (*a*) 10^{-5} g/ml. of carbachol and (*b*) 3.2×10^{-4} g/ml. of acetylcholine on two isolated rat ganglia. The graphs show the development of ganglionic depolarization after adding depolarizing agent to the bath, and its subsidence or reversal on washing (at the break in the abscissae).

Positive potential changes in the kitten ganglion.

Low concentrations of carbachol (10^{-7} or 3.2×10^{-7}) regularly (5 out of 7 experiments) produced a small positive change of the potential difference between the ganglion and the postganglionic trunk of up to 150 or 200 μV (Fig. 8). In experiments where no overt positive shift was seen, the time-course of depolarization was complex, and suggestive of a latent or hidden positive shift, masked by the negative swing (Fig. 9,*b*). The time-

course of depolarization following application of 10^{-6}, and occasionally of higher concentrations, of carbachol was also complex (Fig. 8,*b* and 9). These positive potential changes and potential fluctuations, though small, were sufficiently large to permit the conclusion that they were not spontaneous changes of interelectrode potential, nor the result of disturbances caused by addition of drug (Fig. 8,*c*).

The spatial distribution of the positive potential change usually reflected that of an equivalent-sized depolarization (Fig. 10,*a*). However, in some experiments the region of

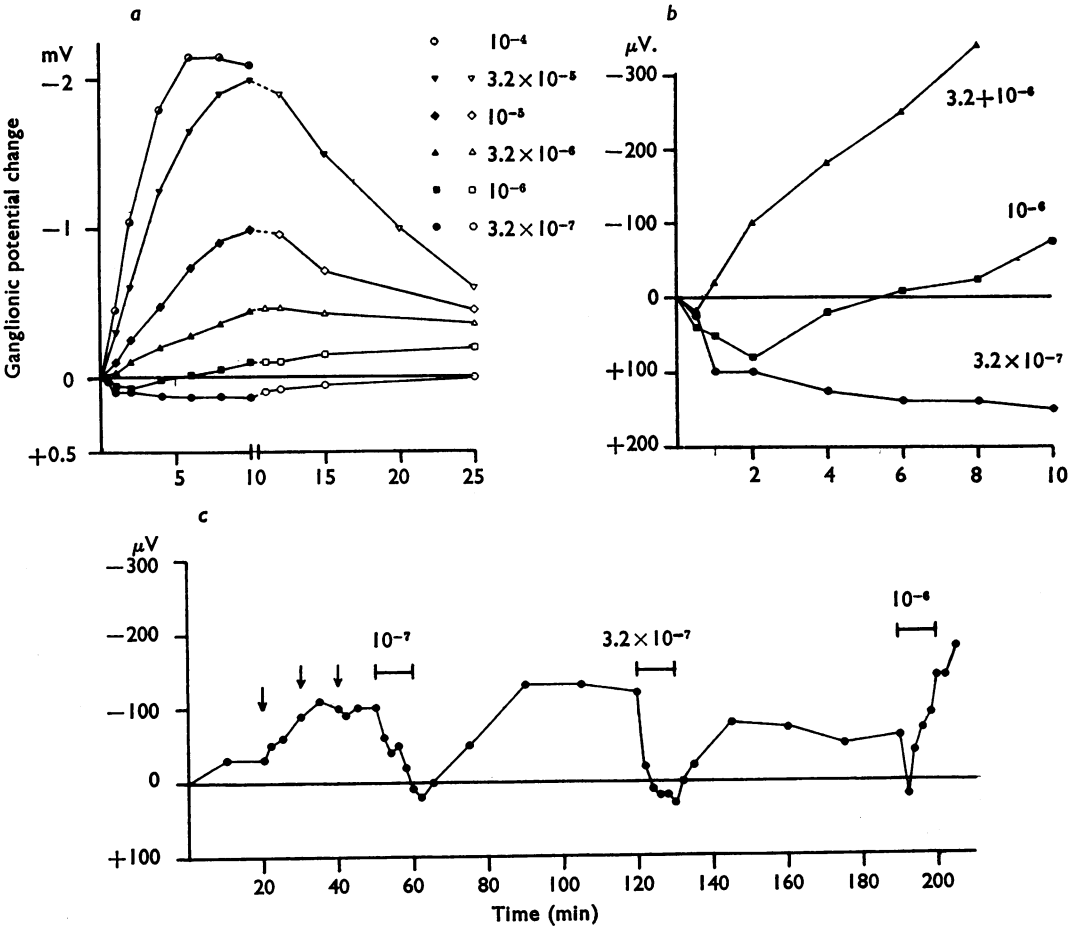


Fig. 8. Positive shifts of the potential difference between the ganglion and postganglionic nerve trunk of an isolated kitten ganglion seen with low concentrations of carbachol. The graphs in (*a*) show the time-courses of depolarization and recovery with a range of concentrations of carbachol. (*b*) Is a larger scale plot of the potential changes seen with the three lowest concentrations of carbachol. In (*c*) the ganglionic potential recorded over a continuous 200-min time period, including part of the initial warming up to room temperature, 3 early changes of bath fluid (at arrows) and 3 applications of carbachol (at bars), is shown. The zero line in (*c*) is drawn arbitrarily from the first recorded potential level: it does not represent the initial demarcation potential, which had already subsided.

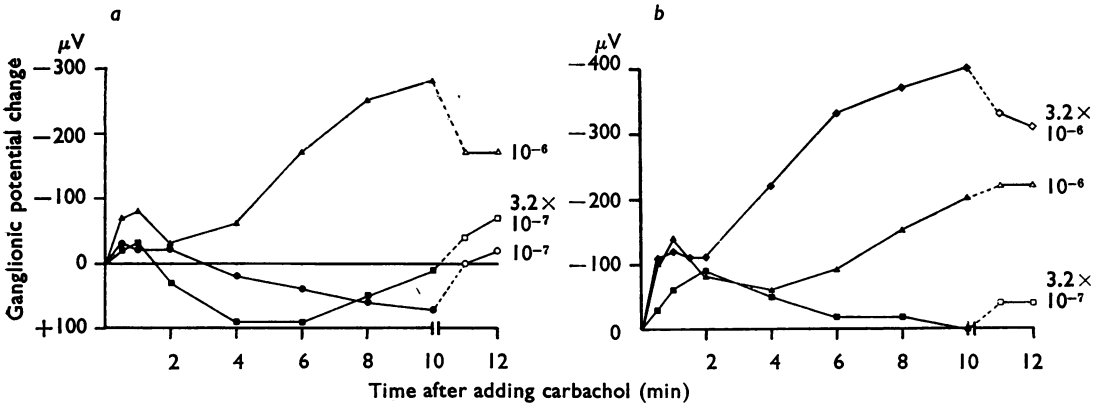


Fig. 9. Complex time-courses of ganglionic potential changes in two isolated kitten ganglia after addition of low concentrations of carbachol.

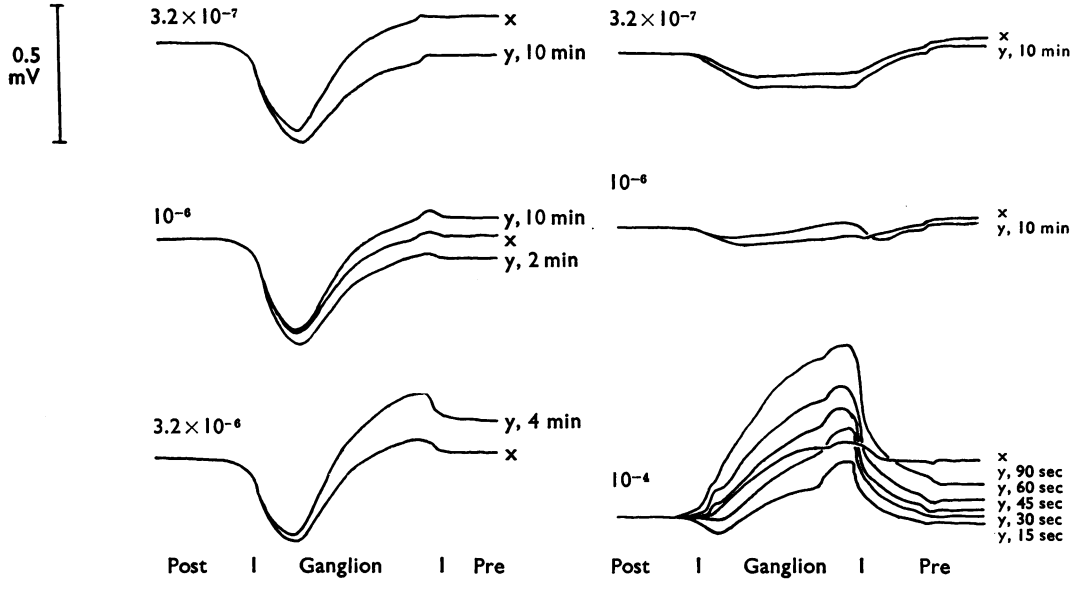


Fig. 10. Tracings of oscilloscope records obtained (x) before and (y) at various times after the addition of carbachol (concentrations indicated) to 2 isolated kitten ganglia. (Negativity upward). In (*a*), negative and positive potential changes induced by carbachol were similar in distribution. In (*b*), the regions of the preparation showing negative and positive potential changes did not coincide.

maximum positive shift clearly did not coincide with the region of maximum depolarization: in this case it was possible to observe a ganglionic negativity at one region of the preparation with a simultaneous ganglionic positivity at another region (Fig. 10,*b*).

A positive potential shift following application of carbachol was seen in one preparation previously treated with 10^{-6} atropine, and in another in the presence of 10^{-3} hexa-

methonium. However, even with very low concentrations of carbachol, the positive shift was not obtained more than two or three times in any one preparation. This lack of repeatability has precluded pharmacological analysis.

Concentrations of methacholine below 10^{-4} also evoked a positive ganglionic potential change, similar in size to that seen with carbachol (Fig. 11 ; 3 out of 3 experiments). In one experiment, methacholine produced a positive change in the presence of 10^{-3} hexamethonium. In all experiments with methacholine, the distribution of the positive potential change obtained with low doses reflected the distribution of ganglionic negativity seen with higher doses (Fig. 11).

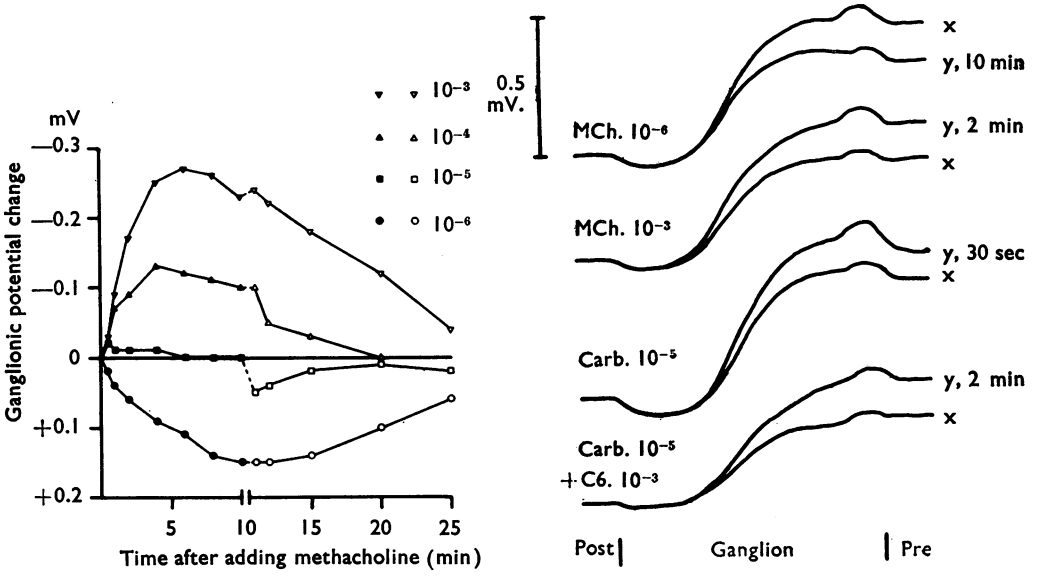


Fig. 11. Ganglionic potential changes produced by different concentrations of methacholine in an isolated kitten ganglion. The graphs on the left show the time-course of the potential changes observed following addition of four concentrations of methacholine to the bath, and (at break in abscissa) wash-out of methacholine. Tracings of oscilloscope records (negativity upwards) obtained before (x) and after (y) addition of methacholine (MCh) and carbachol (Carb) to this ganglion are shown on the right.

No ganglionic positivity has been seen in kitten ganglia with nicotine (10^{-6} to 10^{-4}) or muscarine (10^{-7} to 10^{-5}), but sub-depolarizing concentrations of these agents have not yet been tested. Neither sub-depolarizing nor depolarizing concentrations of carbachol (10^{-9} to 10^{-4}) have been found to evoke positive potential shifts in rat ganglia. Apart from the effect of acetylcholine on the rat ganglion in the presence of high concentrations of hexamethonium (see above), the time-course of the depolarization of the isolated rat ganglion produced by acetylcholine or carbachol showed none of the complexity seen in kitten ganglia.

DISCUSSION

Carbachol, acetylcholine, nicotine, muscarine and methacholine were all capable of depolarizing the isolated kitten superior cervical ganglion. They differed in the magnitude of the depolarization produced and in their susceptibility to antagonism by hexamethonium and hyoscine.

The depolarizing action of *nicotine* accorded with the classical concept of a pure "nicotinic" action, being completely blocked by hexamethonium and unaffected by low concentrations of hyoscine or atropine. Concentrations of atropine of 10^{-5} g/ml. or more depressed the response to nicotine, but since hyoscine did not have a comparable effect until a concentration of 10^{-4} g/ml. was attained, the effect of atropine was probably unspecific.

In contrast to the response to nicotine, depolarization produced by *muscarine* was not reduced by a high concentration (10^{-3} g/ml.) of hexamethonium, but was abolished by 10^{-6} g/ml. of hyoscine. The maximum size of the muscarine depolarization was much less than that obtainable with nicotine, carbachol or acetylcholine. These findings indicate that the depolarizing action of muscarine differed qualitatively from that seen with nicotine. Muscarine depolarization clearly conforms to the accepted criteria of a true "muscarinic" action, rather than to an aberrant "nicotinic" side action. This view is strengthened by the high potency of muscarine as a ganglion-depolarizing agent, concentrations of 10^{-7} g/ml. being maximally effective.

The small depolarization produced by *methacholine* also appeared to be largely of the "muscarinic" type, because it was only slightly impaired by hexamethonium but strongly depressed by hyoscine or atropine. Although high concentrations of methacholine were required to elicit a depolarization (10^{-4} g/ml. or more), these were not much greater than the requisite concentrations of acetylcholine, and might be necessitated because of destruction by ganglionic cholinesterase rather than because of low depolarizing potency of the drug.

The depolarizing action of *carbachol* was complex. It was substantially antagonized by hexamethonium, indicating a strong "nicotinic" action. However, antagonism by hexamethonium was incomplete, even at very high concentrations, and the residual response was blocked by low concentrations of hyoscine. Also, hyoscine alone reduced the effect of carbachol. This suggests that the action of carbachol was partly "muscarinic." This hypothesis is further supported by the observation that the hexamethonium-resistant, hyoscine-sensitive component of the carbachol depolarization was similar in size to the depolarization produced by muscarine. Since the susceptibility of carbachol depolarization to the action of hyoscine appeared to be greater at low concentrations of carbachol than at higher concentrations, it may be that the "muscarinic" potency of carbachol at this site is greater than the "nicotinic" potency.

The action of *acetylcholine* might also be partly "muscarinic," since it was incompletely antagonized by hexamethonium, and more completely blocked by a combination of hexamethonium with hyoscine. However, the "muscarinic" depolarizing action of acetylcholine appeared to be smaller than that seen with carbachol, both on kitten and rat superior cervical ganglia.

There have been several previous reports of "muscarinic" responses of sympathetic ganglia (i.e., atropine-sensitive, hexamethonium-insensitive), including responses to muscarine itself (Ambache, Perry & Robertson, 1956; Konzett & Waser, 1956; Gyermek, Sigg & Bindler, 1963), to methacholine (Takeshige, Pappano, DeGroat & Volle, 1963), to acetylcholine (Takeshige & Volle, 1962, 1963, 1964), and to certain other drugs (Marrazzi, 1939; Ambache, 1949; Franko, Ward & Alphin, 1963; Jones, 1963; Murayama & Unna, 1963). The observations described in the present paper accord with these reports. It is possible to interpret these findings in terms of the presence in the ganglion of separate "nicotinic" and "muscarinic" receptors. Evidence for this hypothesis might become stronger if it could be shown that the two types of receptor were spatially separated, perhaps on separate ganglion cells or different groups of ganglion cells. The records of depolarization obtained with the moving-fluid electrode do not indicate any gross spatial separation. The distribution of the depolarization seen with muscarine and carbachol, or with methacholine and carbachol, were similar. Further, the pattern of potential distribution produced by carbachol was not altered by addition of hexamethonium or hyoscine. There was a difference between the spatial distribution of the depolarizations produced by nicotine and carbachol, the significance of which is not clear. Perhaps more refined recording techniques might show whether there is a physical separation of "nicotinic" and "muscarinic" receptors.

The small positive potential difference between the ganglion and postganglionic trunk evoked by sub-depolarizing concentrations of carbachol and methacholine is of interest in view of the reports by Takeshige *et al.* (1963) and Takeshige & Volle (1964) that these drugs can produce a "hyperpolarization" of the cat superior cervical ganglion *in vivo*. The small size and lack of repeatability of the positive potential change *in vitro* has hindered experimental investigation, so that it is not known how far this is related to the *in vivo* positive potential. On the basis that the complex time-course of depolarization produced by larger doses of carbachol in the kitten ganglion might represent the interaction of negative and positive potential changes, it was hoped to obtain large positive shifts by selectively abolishing the depolarization with hexamethonium (cf. Takeshige & Volle, 1964). As described above, this was not possible even with concentrations of hexamethonium up to 10^{-3} g/ml. Further investigation of the positive potential changes would clearly be desirable.

SUMMARY

1. Drug-induced potential changes in isolated kitten and rat superior cervical ganglia were recorded with a moving-fluid external electrode.

2. In the isolated kitten ganglion, carbachol, acetylcholine and nicotine produced a large (0.5 to 1.5 mV) depolarization, and muscarine and methacholine evoked a small (0.2 to 0.4 mV) depolarization.

3. Hexamethonium (up to 10^{-3} g/ml.) blocked the action of nicotine, and reduced the responses to carbachol and acetylcholine by 55 to 80%, but only slightly impaired the response to methacholine and did not affect the action of muscarine. The hexamethonium-resistant response to carbachol or acetylcholine was greatly reduced or abolished by hyoscine (10^{-6} g/ml.)

4. Hyoscine (10^{-6} g/ml.) abolished the response to muscarine, greatly reduced that to methacholine, partly (30%) reduced that to carbachol, and did not affect those to acetylcholine or nicotine.

5. The responses of rat ganglia to carbachol or acetylcholine were incompletely blocked by hexamethonium (10^{-3} g/ml.), and more completely blocked by hexamethonium (10^{-3}) with hyoscine (10^{-6}).

6. Sub-depolarizing concentrations of carbachol and methacholine produced a small (150 to 200 μV) positive ganglionic potential change in the kitten.

7. It is concluded that drugs may depolarize ganglia by a purely nicotinic action (nicotine), or a purely muscarinic action (muscarine), or by a combination of both nicotinic and muscarinic actions (methacholine, carbachol, acetylcholine). The nature of the positive potential change is not known.

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